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ATTACHMENT I-- FINAL RISK ASSESSMENT OF
ACETOBACTER ACETI

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I. INTRODUCTION

Acetobacter aceti is a benign microorganism that is ubiquitous in the environment, existing in alcoholic ecological niches such as flowers, fruits, honey bees, as well as in water and soil. It has a long history of safe use in the fermentation industry for the production of acetic acid from alcohol. There are no reports in the literature suggesting that *A. aceti* is a pathogen of humans or animals. It also is not considered a plant pathogen. The potential risks to human health or the environment associated with the use of this bacterium in fermentation facilities are low. Since the taxonomy of the genus was recently revised, some older production strains in use for acetic acid production may, in fact, not meet the current taxonomic designation of *A. aceti*.

History of Commercial Use & Products Subject to TSCA Jurisdiction

The history of safe use for this bacterium is predominately for food grade acetic acid (vinegar) production. Members of the genus *Acetobacter* have been used industrially since the 1850's (Edberg, 1991). *A. aceti* has also been reported in the literature as being used for cellulose production for specialty papers or headphones (Anonymous, 1989a, 1989b); however, strains capable of cellulose production are classified as *A. pasteurianus* or *A. hansenii* under the new taxonomic system (De Ley et al., 1984). *A. aceti* is considered a Class 1 Agent under the NIH Guidelines for Research Involving Recombinant DNA Molecules (U.S. Department of Health and Human Services, 1986), and is on the FDA's GRAS (generally recognized as safe) list of microorganisms.

There are a number of TSCA applications for acetic acid. These include manufacturing of acetate rayon, plastics production, rubber production, and photographic chemicals.

II. IDENTIFICATION AND TAXONOMY

A. Overview

Acetobacter aceti is a Gram negative bacterium which is motile by peritrichous flagella. It is obligately aerobic possessing only the ability for respiratory metabolism with no fermentative ability. *A. aceti* does not form endospores. This bacterium is ubiquitous in the environment, existing in soil, water, flowers, fruits, and on honey bees; in essence, wherever sugar fermentation is occurring. *A. aceti* produces acetic acid

from ethanol in alcoholic niches in the environment. Acetate and lactate are oxidized to CO_2 and H_2O by the organism. The optimal temperature for growth is between 25 to 30C, and the Ph optimum between 5.4 to 6.3 (De Ley et al., 1984). *A. aceti* is a common contaminant in all industrial fermentation facilities and is responsible for generating turbidity, ropiness, discoloration, and off-flavors in beer (Kough, 1991).

B. Taxonomy and Characterization

The genus *Acetobacter* is well-defined, although changes in the taxonomy have occurred in recent years. In the 1974 edition of Bergey's Manual of Determinative Bacteriology, there were three species in the genus: (1) *A. aceti* with four subspecies (*aceti, orleanensis, xylinum*, and *liquefaciens*); (2) *A. pasteurianus* with five subspecies (*pasteurianus, lovaniensis, estunensis, ascendens*, and *paradoxus*); and (3) *A. peroxydans*. All these species and subspecies appear on the Approved Lists of Bacterial Names of 1980.

However, the most recent edition of Bergey's Manual of Systematic Bacteriology (De Ley et al., 1984) has reclassified the genus after a numerical analysis was conducted on 177 phenotypic characteristics of organisms in the genus. Presently the genus consists of four species: *A. aceti, A. liquefaciens* (formerly *A. aceti* subsp. *liquefaciens*), *A. pasteurianus* (formerly *A. aceti* subsp. *xylinum* and *orleanensis*), and *A. hansenii*. There are several differentiating characteristics which enable identification of the individual species of *Acetobacter*.

It is important to note that since the genus has recently been revised, older strains in use for acetic acid production may not be correctly classified as *A. aceti*. This risk assessment is for the present-day designation of *A. aceti*.

C. Related Species of Concern

A. liquefaciens, although not considered to be a plant pathogen, has been reported to cause problems in stored fruit. This bacterium does not appear on a Department of Agriculture list of plant pathogens (USDA, 1988), nor is a USDA permit required to have the bacterium in one's possession (Kough, 1991). However, *A. liquefaciens* is capable of producing 2,5-diketogluconic acid which causes pink disease of pineapple (De Ley et al., 1984). This disease is characterized by the discoloration of the tissue to pink which then turns brown with heat during processing (De Ley et al., 1984). Apparently, the fruit itself is unaffected and the browning during processing can be avoided if the fruit is washed prior to processing (Kough, 1991). *A. liquefaciens* may also be involved in rot of apples and pears which has been shown, in some cases, to be associated with

the production of 2,5-diketogluconic acid (Van Keer et al., 1981).

III. HAZARD ASSESSMENT

A. Human Health Hazards

A. aceti has not been reported as a human pathogen. It is ubiquitous in the environment, and therefore, comes in contact with humans on a frequent basis. Its optimum growth temperature is below that of the human body and its optimum pH is below that normally found on the surface of human skin. Due to its close association with sugar breakdown, it is unlikely that this species would form part of the normal bacterial flora of humans (Edberg, 1992). Review articles on the normal flora of the human body did not reveal *A. aceti* (Edberg, 1992).

There are no reports in the literature that *A. aceti* is capable of producing toxins active against humans or animals, nor are there reports of *A. aceti* causing infection in humans or animals (Edberg, 1992). It does not produce enzymes or other extracellular factors normally associated with virulence. There is no reason to suspect that *A. aceti* could acquire or transfer any virulence factors. This bacterium does possess plasmids which are responsible for the production of enzymes used in acetic acid production. These plasmids have been shown to be transferred to other members of the species in the laboratory under optimal conditions. However, there is no evidence of plasmid transfer between strains of *A. aceti* or related species in the environment. Its unique ecological niches are such that it is unlikely that a second recipient or donor microorganism would be present in quantities sufficient for plasmid exchange to occur (Edberg, 1992).

Biochemical characteristics of *A. aceti* virtually preclude it as being a threat to human health. Although it grows well with ethanol as a source of carbon, glucose has been shown to actually decrease the growth rate in culture, especially when other carbon sources were present (O'Sullivan and Ettlinger, 1976). In addition, industrial strains may have been selected so that they do not have the ability to grow on glucose (Weber and Ettlinger, 1971) or so that they utilize very specific amino acids as nitrogen sources. This may result in growth inhibition in the presence of alternate amino acids (O'Sullivan, 1974).

In summary, *A. aceti* has no demonstrated virulence factors. It is not part of the normal flora of human skin or the body and is not expected to survive in a human host for sustained periods of time. The only threat to human health would lie in a massive contamination event in which workers may be exposed to extraordinarily high concentrations of the bacterium, and

perhaps, develop an allergic or immunological reaction. It appears, however, because the bacterium is used for acetic acid production, should such a contamination event occur, the acetic acid would present a greater threat to workers than the bacterium itself. The potential for human virulence is virtually nonexistent for *A. aceti* (Edberg, 1992).

B. Environmental Hazards

1. Hazards to animals

There are no reports in the literature suggesting that *A. aceti* is pathogenic to animals. As previously mentioned, the bacterium does not produce toxins, enzymes, or other extracellular virulence factors normally associated with pathogenicity (Edberg, 1992).

2. Hazards to plants

A. aceti has been reported as being the causal agent of pink disease of pineapple (Kontaxis and Hayward, 1978; Cho et al., 1980). This disease is characterized by a pink discoloration of the fruit which turns brown with heat during processing. The production of the metabolite 2,5-diketogluconic acid is responsible for the discoloration associated with pink disease of pineapple.

These reports suggesting that *A. aceti* is the cause of pink disease of pineapple were published prior to the reclassification of the genus *Acetobacter*. *A. aceti* does not produce 2,5-diketogluconic acid. It is the bacterium now designated as *Acetobacter liquefaciens* (formerly *A. aceti* subsp. *liquefaciens*) which is responsible for this disease through the production of 2,5-diketogluconic acid, not *A. aceti*.

There is another report in the literature of a disease of stored fruit presumably caused by *A. aceti*. This organism, as well as numerous acetic acid bacteria and other bacteria, were reported to cause rot in apples and pears resulting in different degrees of browning (Van Keer et al., 1981). This study involved inoculating apples and pears with 172 strains of bacteria including a variety of acetic acid bacteria from the genera *Acetobacter, Gluconobacter*, and other genera. The entire fruits were inoculated by either 10 mm-deep stab wounds with inoculating needles or by injection to a depth of 10 mm of 0.2 ml of the same pure culture of bacteria with a density of 10^8 cells/ml. Alternatively, sections of the epidermis were removed from the fruit and the bacteria were swabbed over the exposed tissue. Fruits were incubated for two weeks in sterile plastic bags. In other experiments, 4 to 6 mm discs obtained from surface-disinfected fruit were inoculated with the bacterial suspensions by an infected inoculation needle and incubated in petri dishes.

All of the inoculation methods except stab inoculation resulted in rot of apple tissue with most of the acetic acid bacteria tested, including seven strains of what was formerly designated as *A. aceti* subsp. *aceti* and nine strains of other subspecies of *A. aceti*. With the 15 varieties of apples tested, it was concluded that the surface of the fruit must be wounded in order to obtain rot. The three pear varieties tested were shown to be more susceptible to rot, and wounding of the surface was not necessary.

The ability to cause rot of apples and pears as suggested in the above paper may be questionable for bacteria presently designated as *A. aceti*. First, this article was written before the revision of the genus *Acetobacter*, therefore, it is difficult to tell if the strains used would meet the current designation of *A. aceti*.

Second, in order to satisfy Koch's postulates, re-isolations of pure cultures were made from the rotting fruit that had been injected with one strain of *Acetobacter* (species not specified), and two strains of *Gluconobacter* (species not specified). In all cases, 2,5-diketogluconic acid and 2-ketogluconic acid were isolated from the fruit. *A. aceti* can produce 2-ketogluconic acid and 5-ketogluconic acid but does not produce 2,5-diketogluconic acid. The disease of apples and pears is thought to be biochemically similar to pink disease of pineapple (Edberg, 1992). As previously mentioned, pink disease of pineapple is caused by the production of 2,5-diketogluconic acid, which is produced only by *A. liquefaciens*, not *A. aceti* or other species of *Acetobacter*.

Third, although rotting symptoms appeared in fruits that were mechanically inoculated with high concentrations of acetic acid bacteria, rotting symptoms were also demonstrated with 32 strains of bacteria from various genera, some of which are known pathogenic bacteria, but others have no association with plant pathogenicity. The additional bacteria studied included strains from the following genera: *Xanthomonas, Pseudomonas, Frateuria, Escherichia, Agrobacterium, Alcaligenes, Erwinia, Serratia, Paracoccus, Klebsiella, Proteus, Flavobacterium*, and *Chromobacterium*. According to the U.S. Department of Agriculture regulations on biotechnology products under the Federal Plant Pest Act (7 CFR 330, et seq.), no members of the genera *Fratueria, Escherichia, Alcaligenes, Serratia, Parococcus, Klebsiella, Proteus, Flavobacterium*, or *Chromobacterium* are considered plant pathogens, nor are any species of these genera suggested as being plant pathogens according to Bergey's Manual of Systematic Bacteriology (De Ley et al., 1984). Consequently, it appears as though a variety of nonpathogenic bacteria are capable of producing rot symptoms in pears and also in apples when inoculated at high concentrations into the soft tissue below the outer protective epidermal layers.

IV. EXPOSURE ASSESSMENT

A. Worker Exposure

A. aceti is considered a Class 1 Containment Agent under the National Institute of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (U.S. Department of Health and Human Services, 1986). This bacterium also falls under the Class 1 Containment (harmless microorganism) under the European Federation of Biotechnology guidelines (Frommer et al., 1989).

No data were available for assessing the release and survival specifically for fermentation facilities using *A. aceti*. Therefore, the potential worker exposures and routine releases to the environment from large-scale, conventional fermentation processes were estimated on information available from eight premanufacture notices submitted to EPA under TSCA Section 5 and from published information collected from non-engineered microorganisms (Reilly, 1991). These values are based on reasonable worst-case scenarios and typical ranges or values are given for comparison.

During fermentation processes, worker exposure is possible during laboratory pipetting, inoculation, sampling, harvesting, extraction, processing and decontamination procedures. A typical site employs less than 10 workers/shift and operates 24 hours/day throughout the year. NIOSH has conducted walk-through surveys of several fermentation facilities in the enzyme industry and monitored for microbial air contamination. These particular facilities were not using recombinant microorganisms, but the processes were considered typical of fermentation process technology. Area samples were taken in locations where the potential for worker exposure was considered to be potentially greatest, i.e., near the fermentor, the seed fermentor, sampling ports, and separation processes (either filter press or rotary drum filter). The workers with the highest potential average exposures at the three facilities visited were those involved in air sampling. Area samples near the sampling port revealed average airborne concentrations ranging from 350 to 648 cfu/m^3. Typically, the Chemical Engineering Branch would not use area monitoring data to estimate occupational exposure levels since the correlation between area concentrations and worker exposure is highly uncertain. Personal sampling data are not available at the present time. Thus, area sampling data have been the only means of assessing exposures for previous PMN biotechnology submissions. Assuming that 20 samples per day are drawn and that each sample takes up to 5 minutes to collect, the duration of exposure for a single worker will be about 1.5 hours/day. Assuming that the concentration of microorganisms in the worker's breathing zone is equivalent to the levels found in the area sampling, the worst-case daily inhalation exposure is estimated

to range up to 650 to 1200 cfu/day. The uncertainty associated with this estimated exposure value is not known (Reilly, 1991).

B. Environmental and General Exposure

1. Fate of the Organism

A. aceti is widespread in the environment, existing in alcoholic niches such as in flowers, fruit, on honey bees, and in soil and water. Its ubiquitous presence in nature suggests that it is likely to survive if released to the environment.

2. Releases

Estimates of the number of *A. aceti* organisms released during production are tabulated in Table 1 (Reilly, 1991). The uncontrolled/untreated scenario assumes no control features for the fermentor off-gases, and no inactivation of the fermentation broth for the liquid and solid waste releases. The containment criteria required for the full exemption scenario assume the use of features or equipment that minimize the number of viable cells in the fermentor off-gases. They also assume inactivation procedures resulting in a validated 6-log reduction of the number of viable microorganisms in the liquid and solid wastes relative to the maximum cell density of the fermentation broth.

TABLE 1. Estimated Number of Viable *A. aceti* Organisms Released During Production

Release Media	Uncontrolled/ Untreated (cfu/day)	Full Exemption (cfu/day)	Release (days/yr)
Air Vents	$2x10^{8}$ - $1x10^{11}$	$<2x10^{8}$ - $1x10^{11}$	350
Rotary Drum Filter	250	250	350
Surface Water	$7x10^{16}$	$7x10^{10}$	90
Soil/Landfill	$7x10^{18}$	$7x10^{12}$	90

Source: Reilly, 1991

These are "worst-case" estimates which assume that the maximum cell density in the fermentation broth for bacteria is 10^{11} cfu/ml, with a fermentor size of 70,000 liters, and the separation efficiency for the rotary drum filter is 99 percent.
Acetic acid production is also accomplished using a semi-continuous process (De Ley et al., 1984) in which releases are expected to be lower than with a batch production process (LaVeck, 1991).

3. Air

Specific data which indicate the survivability of *A. aceti* in the atmosphere after release are currently unavailable. Survival of vegetative cells during aerosolization is typically limited due to stresses such as shear forces, desiccation, temperature, and UV light exposure. As with naturally-occurring strains, human exposure may occur via inhalation as the organisms are dispersed in the atmosphere attached to dust particles, or lofted through mechanical or air disturbance.

Air releases from fermentor off-gas could potentially result in nonoccupational inhalation exposures due to point source releases. To estimate exposures from this source, the sector averaging form of the Gaussian algorithm described in Turner (1970) was used. For purposes of this assessment, a release height of 3 meters and downward contact at a distance of 100 meters were assumed. Assuming that there is no removal of organisms by controls/equipment for off-gases, potential human inhalation dose rates are estimated to range from 3.0×10^3 to 1.5×10^6 cfu/year for the uncontrolled/untreated scenario and less than that for systems with full exemptions. It should be noted that these estimates represent hypothetical exposures under reasonable worst case conditions (Versar, 1992).

4. Water

The concentrations of *A. aceti* in surface water were estimated using stream flow values for water bodies receiving process wastewater discharges from facilities within SIC Code 283 (drugs, medicinal chemicals, and pharmaceuticals). The surface water release data (cfu/day) tabulated in Table 1 were divided by the stream flow values to yield a surface water concentration of the organism (cfu/l). The stream flow values for SIC Code 283 were based on discharger location data retrieved from the Industrial Facilities Dischargers (IFD) database on December 5, 1991, and surface water flow data retrieved from the RXGAGE database. Flow values were obtained for water bodies receiving wastewater discharges from 154 indirect (facilities that send their waste to a POTW) and direct dischargers facilities that have a NPDES permit to discharge to surface water). Tenth percentile values indicate flows for smaller rivers within this distribution of 154 receiving water flows and 50th percentile values indicate flows for more average rivers. The flow value expressed as 7Q10 is the lowest flow observed over seven consecutive days during a 10-year period. The use of this methodology to estimate concentrations of *A. aceti* in surface water assumes that all of the discharged organisms survive wastewater treatment and that growth is not enhanced by any component of the treatment process. Estimated concentrations of *A. aceti* in surface water for the uncontrolled/untreated and the full exemption scenarios are tabulated in Table 2 (Versar, 1992).

TABLE 2. *A. aceti* Concentrations in Surface Water

Flow	Receiving Stream Flow (MLD*)		Organisms (cfu/l)	
	Mean	7Q10	Mean	7Q10
Uncontrolled/Untreated				
10th Percentile	156	5.60	4.5×10^{8}	1.25×10^{10}
50th Percentile	768	68.13	9.11×10^{7}	1.03×10^{9}
Full Exemption				
10th Percentile	156	5.60	4.5×10^{2}	1.25×10^{4}
50th Percentile	768	68.13	9.11×10^{1}	1.03×10^{3}

*MLD = million liters per day
Source: Versar, 1992

5. Soil

Since *Acetobacter* is a common soil inhabitant, survival in soil would be expected. However, the method of disposal would influence survival. For example, landfilling the organisms would probably not result in long term survival since the anaerobic conditions in landfills would result in cell death. If the organisms were spread out over the surface of the soil, then aerobic conditions would prevail. However, on the soil surface, the bacteria are subject to die-off from UV, temperature, and desiccation. Once in the soil, *A. aceti* populations are expected to decline and reach a steady state population. These releases could result in some human and environmental exposure (LaVeck, 1991).

6. Summary

Although direct monitoring data are unavailable, worst case estimates do not suggest high levels of exposure of *A. aceti* to either workers or the public resulting from normal fermentation operations.

V. INTEGRATION OF RISK

A. Discussion

Acetobacter aceti is ubiquitous in the environment occupying alcoholic niches such as flowers, fruits, honeybees, as well as soil and water. It is found essentially wherever sugarfermentation occurs providing ethanol as a substrate for conversion to acetic acid.

The actual history of the use of *A. aceti* for production of acetic acid from ethanol is not known, however, members of the genus have been used industrially since the 1850's (Edberg, 1992). The main industrial use of *A. aceti* is for the production of vinegar which is not a TSCA application. However, there are TSCA applications for acetic acid including the manufacture of rubber, plastics, acetate fibers, and photographic chemicals.

A. aceti is a benign microorganism. It is included in the Food and Drug Administration's GRAS (generally recognized as safe) list (CFR 21, Parts 170-179, April 1, 1988). It is not pathogenic to humans. Although it often comes in contact with humans due to its widespread presence in the environment, it does not colonize human skin, nor does it inhabit the human body. There are no reports in the literature suggesting any allergic or immunological responses to the bacterium that has been used for decades in fermentation facilities. *A. aceti* does not produce any toxins, enzymes, or virulence factors that usually are associated with pathogenicity. In addition, certain biochemical characteristics of the bacterium such as decreased growth on glucose and growth inhibition in the presence of certain amino acids also lessen the likelihood of human pathogenicity. The potential for human virulence is virtually nonexistent for this species (Edberg, 1992). In addition, worker exposure to the organism is expected to be low (Reilly, 1991).

A. aceti is expected to survive in the environment if released from the fermentation facility. However, exposure to the environment through exhaust gases or liquid wastes are expected to be low under the conditions for inactivation required for this exemption (LaVeck, 1991). Any releases which would occur would not pose any significant ecological hazards, as this microorganism is already ubiquitous in the environment and it is not pathogenic to animals or plants (Kough, 1991). In older literature, prior to the revision of the genus in 1984 (De Ley et al., 1984), *A. aceti* was reported to cause pink disease of pineapple and rot in pears and apples. The former disease is caused by *A. liquefaciens* which was formerly classified as *A. aceti*. Doubt exists on the species identity with the report on rot in apples and pears, since a certain metabolite was found in all infected fruits (Van Keer et al., 1981) but *A. aceti* cannot produce that metabolite (De Ley et al, 1984). In any case, *A. aceti* does not result in typical disease symptoms such as decreased growth or yield loss, and therefore, cannot be considered a true pathogen. In addition, many other nonpathogenic bacteria were also found to cause rot in pears or in apples when mechanically inoculated beneath the surface of the outer protective epidermal layers. Rot of apples and pears caused by *A. aceti* must not occur frequently in nature, as this

is the only citation in the literature reporting this problem. In summary, even if *A. aceti* is released to the environment and is capable of producing rot in pears or rots in apples with damaged epidermal layers, the exposures to orchards or fruit processing plants will probably not be great, or at least not substantially greater than the exposure from naturally-occurring strains of *A. aceti* ubiquitous in the environment.

The only point of concern regarding this microorganism is in regards to the taxonomic revision of the genus *Acetobacter* in the early 1980's. Presently, *A. aceti* is well-defined as a species and is readily distinguished from other *Acetobacter* species. However, older industrial strains of *A. aceti* may not, in fact, meet the current designation of *A. aceti*. Since there are some potential hazards associated with related species in this genus, industrial strains should be verified as being correctly identified as *A. aceti* using the revised taxonomic classification scheme.

B. Recommendation

Acetobacter aceti is recommended for the tiered exemption.

VI. REFERENCES

7 CFR 330, et seq., as amended.

Anonymous. 1989a. Bacteria weave luxury headphones. New Scientist, Mar. 25, 1989, p.31.

Anonymous. 1989b. Biocellulose for specialty papers. Bioprocess Technol. 11:8.

Cho, J.J., A.C. Hayward, and K.G. Rohrbach. 1980. Nutritional requirements and biochemical activities of pineapple pink disease bacterial strains from Hawaii. Antonie van Leewenhoek 46:191-204.

De Ley, J., J. Swings, and F. Gossele. 1984. Genus I. *Acetobacter* Beijerinck, 1898. pp. 268-274. In: N. R. Kreig and J. C. Holt (eds.), Bergey's Manual of Systematic Bacteriology, Vol.1. Williams and Wilkins Co., Baltimore, MD.

Edberg, S.C. 1992. Human health assessment: *Acetobacter aceti*. Unpublished, U.S. Environmental Protection Agency, Washington, D.C.

Frommer, W., B. Ager, L. Archer, B. Brunis, C.H. Collins, R. Donikian, C. Frontali, S. Hamp, E.H. Houwink, M.T. Kuenzi, P. Kramer, H. Lagast, S. Lung, J.L. Mahler, F. Normand-Plessier, K. Sargeant, G. Tuijnenburg Muijs, S.P. Vranch, and R.G. Werner. 1989. Safe biotechnology III. Safety precautions for handling microorganisms of different risk classes. Appl. Microbiol. Biotechnol. 30:541-552.

Gill, D.M. 1982. Bacterial toxins: a table of lethal amounts. Microbiological Reviews 46:86-94.

Kontaxis, D.G. and A.C. Hayward. 1978. The pathogen and symptomology of pink disease of pineapple fruit in the Philippines. Plant Disease Reporter 62:446-450.

Kough, J.L. 1991. Environmental hazard assessment of *Acetobacter aceti* for 5(h)(4) exemption. Unpublished, U.S. Environmental Protection Agency, Washington, D.C.

LaVeck, G. 1991. Exposure assessments of microorganisms considered for 5 (h) (4) exemptions under the proposed biotech rule. Unpublished, U.S. Environmental Protection Agency, Washington, D.C.

O'Sullivan, J. 1974. Growth inhibition of *Acetobacter aceti* by L-threonine and L-homoserine: the primary regulation of the biosynthesis of amino acids of the aspartate family. J. Gen. Microbiol. 85:153-159.

O'Sullivan, J. and L. Ettlinger. 1976. The interaction of four bacteria causing pink disease of pineapple with several pineapple cultivars. Phytopath. 66:369-399.

Reilly, B. 1991. Analysis of environmental releases and occupational exposure in support of the proposed TSCA 5 (h) (4) Exemption. Unpublished, U.S. Environmental Protection Agency, Washington, D.C.

U.S. Department of Health and Human Services. 1986. Guidelines for research involving recombinant DNA molecules; Notice. 51 FR 16958, May 7, 1986.

Van Keer, C., P.V. Abeele, J. Swings, F. Gosselé and J. De Ley. 1981. Acetic acid bacteria as causal agents of browning and rot in apples and pears. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig. C 2:197-204.

Versar, 1992. Screening level exposure assessment of Bacillus species for the 5(h)(4) exemption under the proposed biotech rule. Unpublished, U.S. Environmental Protection Agency, Washington, D.C.

Weber, P. and L. Ettlinger. 1971. The effect of glucose on the utilization of ethanol by a strain of *Acetobacter aceti*. Path. Microbiol. 38:26.